EFFECT OF PATERNAL DEATH ON SPERM VIABILITY IN THE ORANGETHROAT DARTER

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RACIAL VARIATION in gamete function of etheostomatine fishes has been reported by Hubbs (1960). He showed that the fertilizing capacity of sperm exposed to water is greater for orangethroat darters, Etheostoma spectabile, when they are allopatric to species with which they can exchange genes than when they are sympatric. This phenomenon serves as an isolating mechanism to reduce accidental hybridization. The present study tests the hypothesis that there is a difference in sperm viability between an allopatric and a sympatric population of darters when the sperm are retained for as long as 7.5 hours in freshly killed males before exposure to eggs.

Materials and Methods

Orangethroat darters were collected from the San Gabriel River at Georgetown, Texas (allopatric population), and from the north and south forks of the Llano River at Junction, Texas (sympatric with greenthroat darters, Etheostoma lepidum). The males were killed by clipping off their heads with scissors. The dorsal aorta and medulla oblongata were severed, but the coelom was left intact to prevent contamination of the testes. The killed males were held in a water bath at 15° ± 2.0° C. At 0.5-hour intervals, from 0 (control) to 7.5 hours after death, sperm were applied to eggs stripped from living female orangethroat darters. A total of 382 males were tested; 20,241 eggs were used. The stripping and postfertilization techniques used were in accordance with those of Strawn and Hubbs (1956).

Sperm Viability

Sperm viability of each population of darters was measured by the capacity of the sperm to fertilize eggs (tables 1 and 2). These data suggest that sperm decays more rapidly in allopatric males than in sympatric males. After initial tests had indicated that the 0.5-hour delays could be considered as controls, the fertilizations with 0-hour delay were limited to three tests so that gametes could be conserved for the more critical intervals (1.5 to 2.5 hours). Relatively few tests were made at delays greater than 4 hours, as no fertilization occurred in any test in which the delay exceeded 3.5 hours.

To check the possibility that some of the eggs might develop parthenogenically, 2,025 eggs were stripped from 44 females by the same procedure used for normal testing, except that sperm were not introduced. Although some eggs appeared to be clear as long as 6 days after stripping, none formed zygotes.

I observed no significant changes in the hatching or survival percentages, nor any morphological abnormalities in the fry, that were related to the increase in time between death of the male and fertilization. I concluded that paternal

This study was made while the author was a graduate student at the University of Texas.

TABLE 1.--Fertilization, hatching, and early survival of fry of the orangethroat darter when eggs were exposed to sperm from allopatric males

Hours after death of donor	Number of tests	Percentage of tests with some fertilization	Number of eggs fertilized	Percentage fertilized of total eggs used	Percentage of fertilized eggs that hatched	Fry that survived 3 weeks
0	3	100	157	86.8	95.5	87.4
.5	24	100	1,017	59.7	95.4	73.9
1.0	32	84.5	318	18.8	91.5	89.5
1.5	42	50.0	144	6.1	85.4	91.8
2.0	61	32.8	131	3.7	92.1	91.1
2.5	40	10.0	11	.6	91.0	90.0
3.0	35	11.4	12	.6	66.7	87.5
3.5	21	5.1	1	.1	100	100
4.0-7.5	24	0	0	0	0	0

TABLE 2.--Fertilization, hatching, and early survival of fry of the orangethroat darter when eggs were exposed to sperm from sympatric males

Hours after death of donor	Number of tests	Percentage of tests with some fertilization	Number of eggs fertilized	Percentage fertilized of total eggs used	Percentage of fertilized eggs that hatched	Fry that survived 3 weeks
0.5	5	100	319	71.1	94.0	83.6
1.0	12	91.7	275	37.1	96.7	69.6
1.5	19	58.4	102	12.8	86.3	70.5
2.0	23	43.5	58	5.4	98.2	91.2
2.5	29	20.7	33	2.6	100	97.0
3.0	10	20.0	2	.5	100	50.0
3.5	2	0	0	0	0	0

death reduced the number of viable sperm, but did not affect the genetic quality of the spermatozoa that fertilized eggs. Seemingly, the only consequence of using sperm from dead donors was a reduction in the number of offspring.

Sperm Activity

Fifty-two males were killed to make possible observation of sperm activity at 0.5-hour intervals up to 6 hours after death. The semen was stripped from each male separately onto a glass slide, mixed with 1 drop of aged aquarium water, and immediately observed under 430X magnification. Sperm, when motile, were intensely active up to 40 seconds. In the 0- and 0.5-hour-delay samples, most of the sperm were motile; in the 1-hour-delay samples, however, less than 50 percent were active; and in samples taken after longer delays, none were active. Oscillation of individual sperm, without forward movement through the medium, was observed in all samples for 5 to 10 minutes after stripping.

Nonmotile sperm traditionally have been considered nonviable. Rothschild (1962) concluded that:

> ... they move in an obvious way, unlike most other cells, and therefore tell us, visually, if they are alive (always assuming that if they don't move, they are dead) ...

It is now generally accepted that fish sperm show two types of movement. An initial vigorous motion, which moves the sperm toward and through the micropyle, has been observed to last 15 seconds for Salmo fario and 12 seconds for Perca fluviatilis (Pautard, 1962). Upon the cessation of the active swimming phase, the sperm settle down to a period of oscillation if they fail to encounter the stimulating chemical which emanates from the chorion lining of the micropylar canal. Austin (1965) stated that spermatozoa, which show little motility in the surrounding medium, may swim vigorously when they come near the chorion of an egg. Sperm of Salmo gairdneri rapidly lose their motility in ordinary hatchery water, but Terner and Korsh (1963) were able to prolong motility up to 0.5 hour by diluting milt with ovarian fluid.

Although orangethroat darter males that were dead for more than 1 hour did not produce actively motile sperm, males dead for as long as 3.5 hours fathered healthy offspring. My observations indicated that darter sperm, even though so weakened as to be incapable of spontaneous vigorous motility, still have sufficient energy to pass through the micropyle of the egg and fuse nuclei. It is assumed that active swimming is stimulated when the sperm come within the range of ovarian fluid diffusing from the unfertilized eggs.

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A new plastic dog-watering device has been modified for use as a constant-flow fish-treating container.